TEMPERATURE-COEFFICIENTS IN THE ACTIVATION OF STARFISH EGGS BY BUTYRIC ACID.

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INTRODUCTORY.

In experiments performed at Woods Hole during the summers of 1915 and 1916 it was found that unfertilized starfish eggs may be completely activated—so that with normal eggs 95 per cent. or more form blastulæ—by a single exposure to a weak solution of butyric acid (in sea-water or Van't Hoff's solution) at room temperature.[1] To secure this result with a given solution of acid all that is required is that the duration of the exposure should be definite within somewhat narrow limits. Exposures briefer than this optimum cause incomplete activation, which may fail to carry the egg beyond membrane-formation and a few early cleavages; in such cases the activation may be completed and the egg rendered capable of advanced development by a second properly timed exposure to the same solution. Over-exposure directly injuries the eggs and impairs or destroys their power of development. Corresponding to each concentration of acid within a wide range (.0005 *n* to .006 *n*) there was found a well-defined optimum duration of exposure; this duration was approximately inversely proportional to the concentration of acid.

These facts, especially the direct proportionality between the concentration of acid and the speed of the activation-process, indicate that a chemical interaction between the acid and some unknown egg-constituent (probably surface-component), rather than a purely physical effect, is the critical or determinative event in this type of activation. Apparently during the period of exposure this interaction continues uniformly, at a rate determined by the temperature, concentration of acid, and the structural

[1] BIOLOGICAL BULLETIN, 1915, Vol. 28, p. 260; *Journ. Biol. Chem.*, 1916, Vol. 24, p. 233.

conditions in the egg-system, until a certain definite quantity of reaction-product is formed; this is the critical quantity required to enable the egg to continue its development to an advanced stage; and if the reaction is then arrested by returning the eggs to sea-water, activation is found to be complete and development continues normally. If, however, the exposure has been too brief, so that the quantity of reaction-product formed is insufficient, activation is only partial and development ceases at an early stage. Similarly, over-exposure leads to an excessive accumulation of the reaction-product—a condition also unfavorable to development. According to this hypothesis, the activation-process consists essentially in the production of a definite reaction-product, which may be called the activating substance. Since the rate at which the butyric acid takes part in the process is directly proportional to its concentration, it is evident that the interaction has the character of a monomolecular reaction: *i. e.*, the product of the concentration into the time required to produce a given quantity of reaction-product is constant (qt = const.). The quantity of egg-constituent transformed into activating substance in unit time is a direct linear function of the concentration of butyric acid.[1]

Some further test of this hypothesis has seemed desirable, since the objection is possible that the activating effect depends simply upon the entrance of a certain critical quantity of acid into the egg. According to the general law of diffusion, the rate of such entrance would be proportional to the difference in the concentration of acid between medium and cell-interior; hence the time required for the entrance of the activating quantity of acid ought to vary inversely with its concentration in the medium. No inference as to the mode of action of the acid would then be possible from the above facts alone. A possible means of deciding between these alternatives lies in determining the manner in which the rate of activation in a given solution of acid is influenced by change of temperature. The temperature-coefficient

[1] The destruction of an inhibiting substance by combination with the acid might be supposed to underlie the activation effect, but in this case the rate of destruction would not be uniform, but would fall off with the progressive disappearance of the inhibiting substance. All of the evidence indicates that the rate of activation is uniform and a direct function of the concentration of acid.

of reaction-velocities at ordinary temperatures is usually much greater than that of diffusion;[1] hence if the simple rate of diffusion into the egg is the essential factor determining the rate of activation, the effective times of exposure should be only slightly altered by changes of temperature. On the other hand, if the rate of entrance is itself unimportant,[2] and the essential action of the acid consists in forming a chemical compound, the rate of activation should be influenced by temperature in the manner characteristic of chemical relations, *i. e.*, doubled or tripled by a rise of 10°.[3]

EXPERIMENTAL.

In the experiments described below the rates of activation have been determined for a considerable range of temperatures, 2° to 28° (with intervals of 2°), using a single concentration of butyric acid throughout, viz., .006 *n* in sea-water (6 c.c. *n*/10 acid *plus* 94 c.c. sea-water). In each series of experiments eggs from the same lot—usually taken from a single animal to insure uniformity of condition—were exposed simultaneously to this solution at the two (or three) temperatures under comparison. Usually in any single series two temperatures differing by 2° (*e. g.*, 10° and 12°) were chosen; sometimes three temperatures were used, in case this could be done without interfering with manipulation. The procedure was simple: the eggs, after washing thoroughly in sea-water, were placed in two (or three) small

[1] A list of temperature-coefficients of different physico-chemical properties and processes is given in Snyder's paper, *Amer. Journ. Physiol.*, 1908, Vol. 22, p. 309. The values given for diffusion-processes are probably too high; Öholm, working in Arrhenius' laboratory, finds for eight substances, including salts, alkalis and acids (HCl and CH_3COOH) Q_{10} values ranging from 1.19 to 1.28. *Cf.* Öholm, *Zeitschr. f. physik. Chem.*, 1905, Vol. 50, p. 309 (cited in Lewis' "System of Physical Chemistry," Vol. 1, 1916, p. 428).

[2] As would be the case if the rate of entrance were decidedly more rapid than the rate of participation in the activation-process.

[3] This is the usual coefficient for the range of temperatures under consideration in physiological processes. In general the Q_{10} values for chemical reaction-velocities decrease as the temperature rises; for a study of this phenomenon *cf.* Cohen Stuart, *Proceedings of the Royal Society of Amsterdam*, May 23, 1912, p. 1159. Snyder notes that in physiological processes a decrease of Q_{10} at higher temperatures is also frequently found (*Amer. Journ. Physiol.*, 1911, Vol. 28, p. 167). In the activation of starfish eggs by butyric acid, however, the reverse is the case, as will be seen below.

beakers, each containing a thermometer; after the eggs had settled the sea-water was removed as far as possible; then the solutions of butyric acid, at the temperatures chosen for the experiment, were added, first to one beaker, and then, after a short definite interval (*e. g.*, 10 seconds), to the second. The temperature of each beaker was kept constant during the period of the experiment by immersion in a water-bath of the same temperature; and at definite intervals eggs were transferred to finger-bowls containing sea-water. A series of dishes, each containing eggs exposed to .006 *n* butyric acid for a definite length of time (*e. g.*, 1 m., 2 m., 3 m., etc.), was thus obtained for each temperature. The intervals between successive transfers varied from ½ min. at the higher temperatures (22° and over) to 3 or 4 minutes at 8° to 12°. The condition of the eggs in each dish and the proportion developing to larval stages were determined later. The differences between successive members of such a series are usually distinct, and the proportion of favorably developing eggs is always found to increase progressively up to an optimum, after which it declines. The determination of the optimum duration of exposure thus presents no difficulties. As a rule one dish shows distinctly more numerous larvæ than those on either side; but with favorable lots of eggs and brief intervals between the transfers it is often found that at the region of optimum two or even three successive exposures give equally good results.

At temperatures below 8° it is necessary to distinguish between the effects due to the butyric acid, and those produced directly by cold alone. Prolonged exposure to temperatures of 6° and lower has a well-marked activating influence on star-fish eggs.[1] At these temperatures butyric acid solutions were found to act *more* rapidly than at temperatures several degrees higher (8° to 12°); this increase in the rate of activation with fall of temperature indicates the entrance of *cold* as a factor; we have then to deal with a summation of two activating influences, cold and acid. Between 8° and 28° the influence of temperature alone is insufficient for activation. Above this range temperature conditions again become effective; simple exposure to sea-water at 30° and higher for the proper length of time complete activation.[2] There

[1] *Cf.* Greeley, *Amer. Journ. Physiol.*, 1902, Vol. 6, p. 296.

[2] R. S. Lillie, BIOLOGICAL BULLETIN, *loc. cit.*

are thus two ranges of temperature, one below 8°, the other above 29°, exposure to which may induce activation. Eggs may be exposed to sea-water at 28° for 45 minutes without showing any external signs of activation, such as membrane formation, although some latent effect is probably produced.[1] At about 29° definite activation effects begin to appear, and at 30° these are well marked.[2] It is found, however, that at temperatures considerably below the range of heat-activation the action of butyric acid is accelerated to a disproportionate degree by slight rise of temperature; the temperature-coefficient of this action, at first of the order 2–3.5, begins to show decided increase at 20° or even lower, and a rise from 26° to 28° approximately doubles the rate of activation (see Table II). This behavior indicates that at higher temperatures the combination of the acid is facilitated by some secondary change in the egg-system due directly to the temperature. Apparently the structural conditions under which the activating reaction proceeds are modified in a definite manner by a rise in temperature. The indications are that at 30° and higher the structure is so altered that acids formed in the egg itself (*e. g.*, lactic)[3] become free to combine and cause activation; this hypothesis explains why heat-activation and acid-activation exhibit so many features in common, in particular closely similar relations between duration of exposure and degree of activation.[4] The high temperature-coefficients of acid-activation above 20° are thus probably the expression of a super-

[1] This is indicated by the marked acceleration in the rate of acid-activation at or near this temperature. See below, p. 142.

[2] *Loc. cit.*, p. 268.

[3] This acid is formed rapidly in many cells at higher temperatures, as seen in the phenomena of heat-rigor.

[4] *Cf.* my recent paper, BIOLOGICAL BULLETIN, *loc. cit.*, p. 282: also *Journ. Biol. Chem., loc. cit.*, p. 234.

Another characteristic effect which is produced in starfish eggs both by temporary warming and by temporary exposure to butyric acid solutions is the prevention of the maturation-process. Eggs exposed for some minutes to warm sea-water (*e.g.*, 32°), within a few minutes after removal from the animals—*i.e.*, before the dissolution of the germinal vesicle has begun—remain permanently immature (*Journ. Exper. Zoöl.*, 1908, Vol. 5, *cf.* p. 400). The same is true of eggs exposed similarly to butyric acid solution. This identity in the physiological effect produced by the two apparently quite different treatments is a further indication that these high temperatures act by causing the production of acid within the egg.

position of two effects, one a structural alteration due directly to high temperature, the other a chemical combination of the acid with some cell-constituent. The second is the critical event in activation, but its rate is determined not only by the temperature but by the structural conditions in the egg-system.

The action of butyric acid at 6° and lower also represents a summation of two activating influences, cold and acid. I shall deal with this case separately, and shall consider first the temperature-coefficients of the action of butyric acid between 8° and 28°, temperatures which by themselves have no activating effect.

(*a*) *Experiments with Butyric Acid Solution at 8° to 28°.*

It was shown in my two preceding papers[1] that the degree of activation (as indicated by the proportion of favorably developing eggs) resulting from exposure to a given solution of butyric acid at ordinary temperatures increases with increasing time of exposure up to an optimum. Exposures longer than this optimum are injurious and beyond a certain maximum simply cause cytolysis without activation. This rule holds for the action of butyric acid at all temperatures. The interval between the minimum for visible activation (membrane-formation alone) and the optimum decreases rapidly as temperature rises; at 8° this interval is approximately forty minutes, at 18° six to eight minutes, and at 28° less than one minute. A curve relating degree of activation (the percentage of eggs forming blastulæ) to time of exposure may thus be constructed for each temperature. The form of this curve appears to be the same at all temperatures, although as just indicated the time-range which it occupies is shorter at higher temperatures. This behavior is consistent with the foregoing hypothesis that activation depends upon the progressive formation of a reaction-product (acid *plus* egg-substance = activating-substance) to a critical local concentration or quantity, the rate of formation being a function of temperature and concentration of acid.

According to this hypothesis, the interval between the beginning of exposure and the optimum ought to vary with tem-

[1] *Loc. cit.*

perature in accordance with the usual temperature-coefficient of reaction-velocities ($Q_{10} = 2 - 3.5$). The experiments described below show that this is approximately the case for temperatures between 8° and 18°;[1] but with further rise in temperature the Q_{10} values increase at a disproportionately rapid rate, indicating the entrance of some additional factor—probably the direct influence of the high temperature upon the egg-structure, as already indicated. The rate of activation under the influence of heat alone also increases very rapidly with rise in temperature ($Q_{10} = 200 - 400$).[2] Physical changes induced by heat in protein-containing systems—*e. g.*, heat-coagulation or the melting of gelatine gels—show similarly high temperature-coefficients;[3] so that it seems probable that temperatures of the activating range (30° to 38°) produce their effects by altering the physical condition of the structural colloids in the egg-system, and that this change secondarily facilitates or renders possible the chemical interaction upon which activation depends. In order to account for the above rise in the Q_{10} values at temperatures above 20° it seems necessary to assume that as the temperature approaches the region of heat-activation proper, the physical conditions become progressively more favorable for the interaction of butyric acid with the egg-component—*e. g.*, the resistance to the penetration of acid is lessened—so that the reaction is accelerated at a rate higher than can be explained by the influence of temperature upon reaction-velocity alone.

A large number of experiments of the above kind were performed between May 30 and June 28, 1916. During this period at Woods Hole starfish eggs are abundant, and more uniform in

[1] Greeley (BIOLOGICAL BULLETIN, 1903, Vol. 4, p. 129) found with starfish eggs exposed to a mixture of 5 c.c. *n*/10 HCl *plus* 100 c.c. sea-water an approximate optimum at 23° of 5 minutes, at 11° of 15–30 minutes, and at 2° of 45–60 minutes. This indicates for the action of HCl a temperature-coefficient of a similar order. Loeb and Hagedoorn found a similar coefficient for the production of fertilization-membranes in sea-urchin eggs by butyric acid, the physiologically equivalent exposures being twice as long at 10° as at 20° ("Artificial Parthenogenesis and Fertilization," p. 146). The action of hypertonic sea-water shows a similar temperature-coefficient (*cf.* Greeley, *loc. cit.*, pp. 131–133; Loeb, *loc. cit.*, p. 102).

[2] R. S. Lillie, BIOLOGICAL BULLETIN, *loc. cit.*

[3] *Cf.* Schroeder, *Zeitschr. f. physik. Chem.*, 1903, Vol. 45, p. 75; Levites, *Kolloid-Zeitschrift*, 1907, Vol. 2, p. 211; Freundlich, "Kapillarchemie," 1909, p. 416; Chick and Martin, *Journal of Physiology*, 1910, Vol. 40, p. 404, and 1912, Vol. 45, p. 261.

their behavior than later in the season. The general character of the results following simple exposure to butyric acid solutions for varying periods has already been described in my two preceding papers; detailed descriptions of separate series are therefore unnecessary, and the essential results can be presented most concisely in the form of tables. Table I summarizes the results of ten typical series of experiments at temperatures of 8° to 28°. The experiments are divided into two groups, *A* and *B*, one carried out early in June, the other two weeks later. My experience of the last three summers has shown that the effective durations of exposure, both to warm sea-water and to butyric acid solution, are decidedly longer early in the breeding season (up to the middle of June) than later. This is illustrated by the two series of experiments at 18° in Table I.; thus on June 10 the optimum exposure was about 14 minutes; two weeks later (June 24) it had fallen to about 6 minutes.[1] The physiological condition of the eggs used in each group may be regarded as approximately uniform, although certain individual differences of susceptibility are apparent. In both groups the condition of the eggs was good throughout, practically all eggs in the control dishes undergoing normal maturation and developing to larval stages after fertilization; but in the later group the physiologically equivalent exposures are on the average only about half as long as in the earlier. Experiments performed during the intervening period (June 13 to 21) show intermediate conditions (see Table IV).

The estimates of temperature-coefficients for a given temperature-interval (*e. g.*, 8° to 10°) were always made from results obtained with a single lot of eggs; these eggs in nearly all cases came from a single animal. The eggs used in determining each temperature-coefficient thus form a homogeneous group. The total number of series of experiments, each with a separate lot of eggs, performed during the whole period of May 30 to June 28 was twenty-three. Table IV at the end of the paper gives a summary of the character and essential results of all of these experiments.

[1] Compare the optima for the same concentrations of butyric acid given in Table III (June 7–13, 1915) and Table V (July and August, 1915) in my article in *Journ. Biol. Chem., loc. cit.*, pp. 243 and 246.

TABLE I.

A. EXPERIMENTS PERFORMED IN EARLY JUNE.

Lot 2. June 9; 8° and 18°. One starfish. Controls normal.

Temp. of Sol.	Times of Exposure and Percentages of Eggs Forming Blastulæ.												
	3 M.	6 M.	9 M.	12 M.	15 M.	18 M.	22 M.	26 M.	30 M.	35 M.	40 M.	46 M.	54 M.
8°	0	0	0	0	0	0	*ca.* 1%	35–40%	70–80%	**85–90%**	*ca.* **90%**	60–70%	10–20%
18°	0	65–70%	**95%**	*ca.* **90%**	20–30%	0	0	0	—	—	—	—	—

Lot 3. June 9; 10° and 12°. One starfish. Controls normal.

Temp.	3 and 6 M.	9 M.	12 M.	15 M.	18 M.	22 M.	26 M.	30 M.	35 M.
10°	0	0	0	10–15%	40–50%	85–90%	**95%**	*ca.* **95%**	*ca.* 90%
12°	0	<1%	25–35%	80–90%	*ca.* 95%	>**95%**	>**95%**	>90%	20–30%

Lot 4. June 10; 14°, 16°, 18°. One starfish. Controls normal.

Temp.	2 M.	4 M.	6 M.	8 M.	10 M.	12 M.	14 M.	16 M.	18 M.	20 M.	22 M.	24 M.	26 M.
14°	0	0	0	0	<1%	10–15%	10–15%	25–30%	40–50%	50–60%	**70–80%**	**70–80%**	*ca.* 70%
16°	0	0	0	*ca.* 1%	*ca.* 5%	25–35%	50–60%	70–80%	**75–85%**	**75–85%**	70–80%	——	——
18°	0	0	*ca.* 1%	25–35%	50–60%	70–80%	**80–90%**	80–90%	25–35%	——	——	——	——

Lot 6. June 12; 8° and 10°. Two starfish. Controls normal.

Temp.	4 M.	8 M.	12 M.	16 M.	20 M.	24 M.	28 M.	32 M.	36 M.	40 M.	44 M.	48 M.
8°	0	0	0	*ca.* 1%	*ca.* 2–3%	5–10%	40–50%	70–80%	**80–90%**	**80–90%**	65–75%	5–10%
10°	0	0	*ca.* 1%	10–15%	30–40%	60–70%	80–90%	**85–90%**	**85–90%**	70–80%	50–60%	10–15%

Lot 7. June 12; 20°, 22°, 24°. Two starfish. Controls normal.

Temp.	1 M.	2 M.	3 M.	4 M.	5 M.	6 M.	7 M.	8 M.	9 M.	10 M.
20°	0	0	<1%	*ca.* 5%	25–30%	*ca.* 50%	60–70%	*ca.* 90%	*ca.* **95%**	*ca.* 90%
22°	0	0	*ca.* 10%	30–40%	70–80%	*ca.* **95%**	*ca.* **95%**	*ca.* 90%	——	——
24°	1%	30–35%	*ca.* 90%	*ca.* **95%**	40–50%	10–15%	——	——	——	——

B. Experiments Performed in Late June.

Lot 23. June 26; 8° and 10°. One starfish. Good control (fair number of eggs fail to maturate).

Temp.	3 M.	6 M.	9 M.	12 M.	15 M.	18 M.	21 M.	24 M.	27 M.	30 M.	33 M.	36 M.
8°	0	0	<1%	5–10%	20–30%	30–40%	*ca.* 50%	**50–60%**	30–40%	5–10%	<1%	0
10°	pr. 0	<1%	*ca.* 1%	5–10%	55–60%	**65–75%**	**65–75%**	25–35%	15–20%	10–15%	——	—

Lot 22. June 26; 12°, 14°, 16°. One starfish. Controls normal.

Temp.	2 M.	4 M.	6 M.	8 M.	10 M.	12 M.	14 M.	16 M.	18 M.	20 M.	22 M.
12°	*ca.* 5%	15–20%	25–35%	**80–90%**	**70–80%**	**70–80%**	*ca.* **75%**	50–60%	40–50%	*ca.* 10%	0
14°	10–15%	*ca.* 50%	60–70%	**60–70%**	**80–85%**	*ca.* 50%	*ca.* 10%	*ca.* 1%	0	0	—
16°	30–35%	*ca.* 50%	**80–85%**	**70–80%**	15–20%	2–3%	0	0	0	——	—

Lot 21. June 24; 18°, 20°, 22°, 24°. One starfish. Controls normal.

Temp.	½ M.	1 M.	1½ M.	2 M.	2½ M.	3 M.	3½ M.	4 M.	5 M.	6 M.	7 M.	8 M.	9 M.
18°	—	0	—	<1%	—	15–20%	—	60–70%	**80–85%**	**80–90%**	70–80%	60–70%	25–35%
20°	—	0	—	15–20%	—	*ca.* **95%**	—	*ca.* **90%**	**80–90%**	35–45%	*ca.* 1%	0	
22°	0	0	35–40%	75–85%	**75–85%**	**80–90%**	60–70%	—	—	—	—	—	—
24°	0	5–10%	*ca.* **90%**	*ca.* **90%**	50–60%	<1%	—	—	—	—	—	—	—

Lot 17. June 22; 24°, 26°. One starfish. Controls normal.

Temp.	½ M.	1 M.	1½ M.	2 M.	2½ M.	3 M.
24°	0	25–35%	80–90%	**95%**	40–50%	10–15%
26°	<1%	**70–80%**	**70–80%**	10–15%	0	0

Lot 20. June 24. 26°, 28°. One starfish. Controls normal.

Temp.	½ M.	1 M.	1½ M.	2 M.
26°	5–10%	**80–90%**	30–35%	0
28°	**80–90%**	pr. 0	0	0

In Table I the figures represent the approximate percentages of eggs forming free-swimming blastulæ with each exposure. The estimates of percentages were made in watch-glasses under a low power; a large number of eggs were used in making each estimate, collected at random from the bottom and sides of the dish. The probable error of such estimates is assumed to be about 5 per cent.; hence the values are given as approximations; this procedure appears to be as accurate as the conditions permit. There is always a clearly defined optimum in such a series; but often two and sometimes three successive dishes near the optimal region show equally good conditions, especially if the intervals between successive exposures are brief. In such cases the optimum used in calculating temperature coefficients is the arithmetical mean of the favorable exposures. For convenience in supervision the optima are printed in heavy type.

Table II summarizes the results of the above experiments, and gives the temperature-coefficients for each series. The coefficients are given as the ratios between the velocities at two temperatures 10° part (Q_{10} values), in accordance with the usage customary in physiological literature. It is assumed that the velocity increases through the entire interval of 10° at the same proportional rate as through the observed interval of 2°; the value of Q_{10} is then $(T_n/T_{n+2})^5$, T_n and T_{n+2} being the observed optimal times of exposure at the two temperatures.[1] Each value of Q_{10} is derived from observations made upon the same lot of eggs at the two temperatures.

In each group the temperature-coefficient is seen to increase steadily as the temperature rises. The two groups are best compared if the Q_{10} values are placed side by side as in Table III.

Further experimental data are given in Table IV at the end of the paper, which summarizes the results of all of last season's experiments. In this table the experiments for each temperature are grouped together; the decrease in the effective times of ex-

[1] The temperature-coefficient as thus expressed is obtained from any pair of observations by the formula,

$$Q_{10} = \left(\frac{V_{t_1}}{V_{t_2}}\right)^{\frac{10}{t_1 - t_2}}$$

V_{t_1} and V_{t_2} being the velocities observed at the two temperatures t_1 and t_2.

TABLE II.

GROUP *A*. (June 9–12).

Number and Date of Series.	Temperatures of Solution and Optimum Exposures.	Mean Optimum.	Value of Q_{10}.
2. June 9.....	8°:35–40 min. 18°: 9–12 min.	37.5 m. 10.5 m.	3.55
6. June 12.....	8°:36–40 min. 10°:32–36 min.	38 m. 34 m.	1.75
3. June 9.....	10°:26–30 min. 12°:22–26 min.	28 m. 24 m.	2.2
4. June 10.....	14°: 22–24 min. 16°: 18–20 min. 18°: *ca.* 14 min.	23 m. 19 m. 14 m.	2.6 4.65
7. June 12.....	20°: 9 min. 22°: 6–7 min. 24°: 4 min.	9 m. 6.5 m. 4 m.	5.0 11.6

GROUP *B*. (JUNE 22–26).

23. June 26.....	8°: 21 –24 min. 10°: 18 –21 min.	22.5 m. 19.5 m.	2.0
22. June 26.....	12°: 8 –14 min. 14°: 8 –10 min. 16°: 6 – 8 min.	11 m. 9 m. 7 m.	2.7 3.55
21. June 24.....	18°: 5 – 6 min. 20°: 3 – 5 min. 22°: 2½– 3 min. 24°: 1½– 2 min.	5.5 m. 4 m. 2.75 m. 1.75 m.	5.0 6.4 9.5
17. June 22.....	24°: 2 min. 26°: 1 – 1½ min.	2 m. 1.25 m.	10.5
20. June 24.....	26°: *ca.* 1 min. 28°: *ca.* ½ min.	1 m. .5 m.	32.0

TABLE III.

Interval.	Values of Q_{10}. *A*. June 9–12.	*B*. June 22–26.
8°–10°	1.75	2.0
10°–12°	2.2	
12°–14°		2.7
14°–16°	2.6	3.55
16°–18°	4.65	
18°–20°		5.0
20°–22°	5.0	6.4
22°–24°	11.6	9.5
24°–26°		10.5
26°–28°		32.

posure with rise of temperature, and the tendency to a shortening of the reaction-time as the season advances, are both shown clearly. The date of each experiment is given, and each lot of eggs is designated by number so as to render possible comparison between different lots. It will be noted that in general the temperature-coefficients between 8° and 18° are of the usual order of chemical reaction-velocities, but that above 18° they increase rapidly. The significance of this increase has already been considered.

(*b*) *Experiments at Temperatures Below 8°.*

In these experiments the sea-water and solutions used were first cooled at the required temperature, and then added to the beakers containing the eggs as before. The temperatures were kept constant during the period of the experiment by immersing the beakers in battery-jars containing cold water, together with a sufficient quantity of chopped ice to keep the temperature at the desired point. With proper care the fluctuations of temperature are slight under these conditions—usually less than a degree on either side of the temperature chosen, an approximation sufficient for the purpose of these experiments.

Table V gives the results of three series of experiments with a single lot of eggs at 2°, 4°, and 6°. The eggs were placed, shortly before the separation of the first polar body, in the solution of butyric acid (.006 *n* in sea-water) at the three temperatures, and portions were returned to normal sea-water at room temperature after exposures varying from 5–65 minutes.

TABLE V.

JUNE 13, 1916. EGGS FROM TWO STARFISH. CONTROLS SHOW NORMAL MATURATION AND NORMAL DEVELOPMENT AFTER FERTILIZATION.

Temperature.	Times of Exposure and Percentage of Eggs Forming Blastulæ.										
	5 M.	10 M.	15 M.	20 M.	25 M.	30 M.	35 M.	40 M.	45 M.	50 M.	55 and 60 M.
Ser. *A*, 2°..	*ca.* 1%	2–3%	1–2%	3–4%	15–20%	35–40%	25–35%	0	0	0	0
Ser. *B*, 4°..	<1%	*ca.* 5%	5–10%	15–20%	35–40%	55–60%	20–25%	0	0	0	0
Ser. *C*, 6°..	3–4%	1–2%	*ca.* 5%	15–20%	30–40%	*ca.* 90%	30–40%	*ca.* 5%	*ca.* 1%	0	0

With exposures of 35 minutes and longer a large proportion of the eggs undergo complete cytolysis ("ghosts" next day), especially in Series *A* (*A* > *B* > *C*).

In each series the eggs show an optimum of activation with an exposure of about 30 minutes. The differences between the three series are not great; in each series over-exposure (35 minutes and longer) is followed by the complete breakdown and disintegration of a large proportion of eggs; by next day nothing is left of these eggs but a thin structureless residue ("ghosts"). This dissolution is much more complete than that resulting from moderate over-exposure to butyric acid at higher temperatures. It will be noted that the proportion of eggs developing favorably with the optimum exposure is highest at 6° and lowest at 2°; *i. e.*, the destructive effect is greater the lower the temperature.

The higher rate of activation in these experiments, as compared with that observed at slightly higher temperatures (8° to 10°), is almost undoubtedly the expression of a summation of the two separate effects of cold and butyric acid. This is indicated by the results of exposure to normal sea-water at these temperatures. On June 14 eggs were exposed, under the same conditions as above, to normal sea-water at 2°, 4°, and 6°. The result was a typical though partial activation in each series; a large majority of eggs—not all—formed membranes and underwent irregular fragmentation and breakdown without further development. In each series a considerable proportion of eggs (about one third to one fourth) remained apparently unaffected, and later underwent the usual postmaturational coagulation without membrane-formation or development. Several other similar experiments yielded similar results. The above durations of exposure are evidently too brief for complete activation. With longer exposures (about 6 hours) a few eggs may form larvæ under these or similar conditions.[1] Evidently low temperature alone may initiate the activation-process; hence with cold and butyric acid combined the rate of activation may be more rapid than with butyric acid at the higher temperatures of 8° and 10°, which by

[1] In Greeley's experiments (*loc. cit.*, 1902) the best results were obtained when the eggs were exposed, beginning three to four hours after the completion of maturation, for six to nine hours to temperatures of 4°–5°. The highest proportion of larvæ obtained was 20 per cent.; usually only 1–2 per cent. of eggs formed larvæ. The period after maturation is completed is in general unfavorable for activation in starfish eggs.

themselves have no activating effect upon these eggs. Eggs exposed on June 15 to sea-water at 8° and 10° for periods ranging from 5 to 50 minutes showed no signs of activation.

The rate of activation under the influence of low temperature alone is very gradual. Out of eight additional series of experiments with cold normal sea-water (1° to 5°), performed at different times up to July 19, and in which the longest exposures were respectively $4\frac{1}{2}$, 8, 6, $7\frac{3}{4}$, 8, 7, 7, and 6 hours, only one yielded any swimming larvæ; in this series (July 2) about one per cent of the eggs formed blastulæ after exposures to sea-water at 1° to 3° for 7 hours (see Table VI). In the other series the only evident effect was the formation of fertilization-membranes in a variable proportion of eggs, followed by irregular changes of form and breakdown; with the longer exposures a certain proportion of eggs cleaved in some experiments, but none developed further.

It had previously been observed that the addition of alcohol caused a decided acceleration of the activation process in butyric acid solution or warm sea-water. For example, in sea-water containing 3 or 4 volumes per cent. C_2H_5OH the exposure to .003 *n* butyric acid required to cause a majority of eggs to form blastulæ was shortened from 7 or 8 minutes to about 3 minutes.[1] This result suggested the possibility that the rate of activation at low temperatures might similarly be increased by the presence of alcohol. The experiments described in Table VI show that this is the case.

It is clear that activation at low temperatures as well as at high temperatures is favored by the presence of alcohol. In solutions of the above concentrations, alcohol, acting alone at ordinary temperatures, has no evident effect; eggs exposed on June 29 to 4 vols. per cent. alcohol at the three temperatures 18°, 22°, and 25°, for periods ranging from 2 to 24 minutes, showed neither membrane-formation nor development. The favorable effect in the above series is thus due not to the direct activating influence of the alcohol, but to a facilitation or acceleration of the activation-reaction, which takes place spontaneously though slowly at these low temperatures. It seems probable

[1] R. S. Lillie, *Jour. Biol. Chem., loc. cit.*, p. 246, footnote.

that the alcohol alters the colloidal substratum in such a manner as to increase the diffusion-rates of the interacting substances; at least some change in the structural or catalytic conditions determining reaction-velocities is indicated. The terms facilitation and sensitization merely describe or classify the effect without throwing any further light upon its physico-chemical nature.

TABLE VI.

July 1. Eggs from one starfish were placed in (*A*) cold normal sea-water, and (*B* and *C*) cold sea-water containing respectively 3 and 4 vols. per cent. ethyl alcohol. The eggs were kept in flasks immersed in an ice-water bath; the temperature of each flask was about 3° (varying between 2° and 4°). At intervals of 2, 4, 6, and 8 hours eggs were transferred to sea-water at room temperature.

The majority of control eggs (*i. e.*, unfertilized and sperm-fertilized eggs at room temperature) showed normal behavior. Results were as follows:

Times of Exposure.	*A*. Normal Sea-water.	*B*. 3 V. % C_2H_5OH.	*C*. 4 V. % C_2H_5OH.
1. 2 h.	Most eggs form membranes and break down.	All eggs form membranes; 4 or 5 blastulæ found.	All form membranes; a few blastulæ.
2. 4 h.	Same as *A* 1; no blastulæ.	A few blastulæ.	*Ca.* 4–5% of eggs form blastulæ.
3. 6 h.	No blastulæ.	A few blastulæ.	*Ca.* 15–20% blastulæ.
4. 8 h.	No blastulæ; some cleavages.	Blastulæ more numerous; *ca.* 1–2%.	*Ca.* 30–35% blastulæ.

The results of a similar experiment on July 2, with 4 and 5 vols. per cent. alcohol, were as follows:

Exposures.	*A*. Normal Sea-water.	*B*. 4 V. % C_2H_5OH.	*C*. 5 V. % C_2H_5OH.
1. 1 h.	20–25% of mature eggs form membranes; one blastula found.	All mature eggs form membranes; 10 to 15% blastulæ formed.	Membranes in all mature eggs; *Ca.* 10–15% blastulæ.
2. 3½ h.	Like A1; a few blastulæ.	*Ca.* 25–35% blastulæ.	*Ca.* 35–45% blastulæ.
3. 4½ h.	Like *A* 2.	*Ca.* 30–40% blastulæ.	*Ca.* 70–80% blastulæ.
4. 7 h.	Blastulæ more numerous; *ca.* 1%.	*Ca.* 10–15% blastulæ.	*Ca.* 30–40% blastulæ.

The essential question is why low temperatures should so alter the conditions within the cell as to render possible the activation-reaction. That cold may cause definite structural changes in many cells, leading to cytolysis or other characteristic effects, is well known. Such effects are often found at temperatures well above the freezing-point, *e. g.*, the fatal action of sub-normal

temperatures upon tropical marine animals[1] or warm-blooded vertebrates. Greeley describes protoplasmic condensation and loss of water in *Stentor* and other organisms as a result of prolonged exposure to low temperatures (6° and lower in most of his experiments), and he calls attention to various resemblances between the effects produced by cold and by hypertonic solutions.[2] It does not seem probable, in the light of more recent knowledge, that the activation following the exposure of starfish eggs to cold and to hypertonic sea-water respectively can be referred directly to the same cause, namely, loss of water, as Greeley supposed; but the evidence that a lowering of temperature below a certain critical point produces definite structural alterations has an obvious bearing on the present problem. In the case of high temperatures (above 30°) the activating influence is probably to be referred to structural changes in the protoplasmic system, as the temperature-coefficients indicate; and the same appears to be true for cold. Changes in the physical condition of the structural colloids—*e. g.*, gelation, dehydration, altered aggregation-state—may alter locally the permeability or other properties of the protoplasmic system (*e. g.*, of membranes or other barriers to diffusion), and thus render possible interactions which are not possible at ordinary temperatures. This seems to be the most consistent general explanation for the fact that in the starfish egg temperatures both above and below a certain range, 8° to 28°, may induce activation. This range may be regarded as corresponding to the range of stability of the structures concerned.

General Discussion.

The phenomena under consideration in the present paper exhibit many features in common with those accompanying or conditioning cytolysis in other cells. Cytolysis in weak solutions

[1] The tropical medusa Cassiopea shows an interesting parallel to the conditions in starfish eggs. The animals may be cooled to 9.5° and recover, if immediately returned to sea-water at the normal temperature of 29°; but if cooled to 7° or 8° there is no recovery; some irreversible change is produced and the tissues disintegrate on return to warm water (E. N. Harvey, "Effect of Different Temperatures on *Cassiopea*," Carnegie Institution Publications, 1910, No. 132, p. 32).

[2] Greeley, *Amer. Journ. Physiol.*, 1902, Vol. 6, p. 122; Biological Bulletin, 1903, Vol. 5, p. 42.

of acids and bases, as well as under the influence of high temperatures, is a phenomenon of general occurrence, and its conditions resemble closely those already described as determining the rate and character of activation in starfish eggs. Both processes require time, and end in structural alterations of a definite kind; in the egg membrane-formation is the first visible change, which may be followed by development; in cytolysis the essential effect appears to be an alteration of the surface-layer or plasma-membrane of the cell; this structure loses its normal insulating or semi-permeable properties, with the result that the diffusible cell-constituents pass into solution in the surrounding medium; the cell then disintegrates. The physico-chemical conditions of cytolysis have been investigated most completely in red blood-corpuscles, and exact data are available with reference to the influence of both the concentration of the hæmolytic agent and of temperature upon the rate of hæmolytic action. Many facts indicate that a change similar in kind to that underlying cytolysis, only reversible and of brief duration, forms a primary feature of both normal and artificial activation. According to Loeb an incipient or superficial cytolysis is the first stage in the chemical activation of sea-urchin eggs;[1] and this view has been substantiated by a large number of investigations, which have shown that cytolytic agents of the most varied kind, chemical and physical, may cause parthenogenesis.

All of this evidence indicates that the initial change in the activation of the resting egg is *superficial*, and associated with a general increase in the permeability of the egg-surface to water-soluble substances and water.[2] One general consequence of such a change is electrical depolarization; and from the analogy with the phenomena of stimulation it seems probable that this electrical variation, as such, forms the critical change of condition by which the course of the metabolic processes in the egg is so modified as to initiate development. Structural and metabolic alterations go hand in hand and mutually influence each other, as has lately been especially emphasized by Child;[3] and the ini-

[1] J. Loeb. "Artificial Parthenogenesis and Fertilization," Chapter 17.

[2] *Cf.* my recent paper in *Amer. Journ. Physiol.*, 1916, Vol. 40, p. 249.

[3] C. M. Child, "The Regulatory Processes in Organisms," *Journal of Morphology*, 1911, Vol. 22, p. 173; *cf.* also "Senescence and Rejuvenescence," University of Chicago Press, 1915, especially Chapters 1 and 2.

tiation of development in unfertilized eggs through a slight change in the physical state of the surface-film offers perhaps the clearest illustration of this principle. The general conditions of cytolysis have thus an intimate bearing upon the question of the nature of the initial process in activation.

Arrhenius and Madsen have studied by exact methods the conditions of hæmolysis in weak solutions of acids and bases.[1] Their experiments with ammonia offer perhaps the closest analogies to the above experiments with starfish eggs. The rate of hæmolysis has been found to be directly proportional to the concentration of ammonia, and to increase at a rapid rate with rise of temperature. They conclude that the hæmolytic effect depends upon a chemical reaction of the monomolecular class. Evidently this reaction must proceed to a certain stage before the corpuscles are sufficiently altered to release their hæmoglobin. The times required to produce at constant temperature (0°) a definite degree of hæmolysis by different concentrations of ammonia are given in the following table.[2]

TABLE VII.

Concentration of NH_3 (n).	Time of Action Required for a Given Per Cent. of Haemolysis (in Minutes).			
	10 %.	20 %.	30 %.	40 %.
.001 n	26 m.	35 m.	44 m.	53 m.
.00227	10 m.	15 m.	18 m.	23 m.
.00435	5.5 m.	9 m.	12 m.	14 m.
.0075	....	4 m.	6.2 m.	8 m.

The numbers in the vertical columns show that the rate of hæmolytic action is directly proportional to the concentration of ammonia (qt = const.). In the horizontal columns the relations between time of action and degree of hæmolysis are shown for each concentration of ammonia; these relations are also similar to those between time of exposure and degree of activation in starfish eggs; for example, in the experiment of June 10 at 14° (Table I) the proportion of fully activated eggs with exposures of 14, 16, 18, and 20 minutes were respectively

[1] *Cf.* Arrhenius, "Immunochemistry," Macmillans, 1907, Chapter 4; also "Quantitative Laws in Biological Chemistry," London, Bell and Co., 1915.

[2] "Immunochemistry," p. 101; "Quantitative Laws," p. 64.

10–15 per cent., 25–30 per cent., 40–50 per cent., and 50–60 per cent.; compare (*e. g.*) the proportion of corpuscles hæmolyzed by .00227 *n* NH_3 in 10, 15, 18 and 23 minutes, viz., respectively 10, 20, 30, and 40 per cent. The influence of temperature upon the rates of the two processes is also of the same general kind, although certain differences are seen; in both cases the average value of the temperature-coefficients is greater than that of typical chemical reaction-velocities; but in hæmolysis the variability of the coefficient with change of temperature is less, and its value decreases with increase in the effective time of action.[2] The agreements, however, are sufficiently close to indicate that the same kind of process lies at the basis of both effects,—consisting apparently in the progressive combination of the acid or base with some cell-constituent until a certain critical quantity or local concentration of reaction-product is formed. Arrhenius also infers a chemical binding of the hæmolyzing acid or base to some substance in the erythrocytes.

In general we may conclude that in both cytolysis and the activation of the egg-cell the characteristic effect depends primarily upon the formation and accumulation of a reaction-product whose presence is the cause or condition of some definite physical change of state or structural alteration in the cell-system, especially in the surface-layer. In the egg-cell the structural conditions under which the normal metabolic reactions take place are modified, and the course of metabolism is changed; development then proceeds. In either type of cell, if an insufficient quantity of the activating substance is formed—as by too brief exposure—the effect is incomplete or fails to appear; thus in order to liberate hæmoglobin from erythrocytes by means of a given solution of ammonia at a constant temperature, a certain minimal time of exposure is required; with briefer exposures no visible effect is produced. We have seen that in the starfish egg exposures briefer than the optimum may produce visible effects (membrane-formation, imperfect cleavage, etc.) indicating a partial degree of activation; in the case of hæmolysis it is also to be assumed that brief exposures cause a partial cytolytic effect, which however is insufficient to liberate hæmoglobin and

[2] "Immunochemistry," pp. 107 *seq.*; "Quantitative Laws," pp. 65 *seq.*

hence escapes observation. The egg differs from the erythrocyte in showing definite evidence of an incomplete reaction, but in other respects the conditions in the two kinds of cell are alike.

All of the conditions indicate that in activation as in cytolysis the primary reaction takes place at the cell-surface. This view is confirmed by the promptness with which the course of the activation-process in butyric acid solution can be arrested at any desired stage by a return to sea-water. It seems clear that combination of the acid with some surface-component is concerned; a difference of half a minute or less in the time of exposure to .006 *n* butyric acid, especially at higher temperatures (24° and over) may make all of the difference between incomplete and complete activation, or between activation and destruction of the egg. In its prompt initiation in the solution of acid and its equally prompt arrest by return to sea-water the activation-reaction resembles closely the type of response characteristic of sensory structures like taste-buds or other chemical receptors in normal chemical stimulation. Crozier has recently reached the conclusion that in this case also stimulation is the result of a chemical surface-interaction.[1] Here again the existence of a far-reaching parallelism between the process of stimulation and the activation of the egg-cell is indicated. It appears probable that advance in the general physiology of stimulation will furnish the key to the interpretation of the activation-process.

The high temperature-coefficients of heat-cytolysis and of heat-activation in eggs indicate clearly that alterations of certain structural colloids of the cell lie at the basis of these effects. Reactions are thus enabled to take place in the egg which in some manner lead to the initiation of development. The initial chemical reaction in heat-activation is probably simple in character and similar to that of acid-activation, as already suggested. Apparently this reaction forms the condition of some definite structural change in the egg-system; this change is followed by the series of developmental changes.

It is possible that an autolytic process may form the first step

[1] *I. e.*, the time required for stimulation is much shorter than that required for visible penetration of the acid into the cell-interior; *cf.* W. J. Crozier, *Jour. Biol. Chem.*, 1916, Vol. 24, p. 255; *cf.* pp. 270 *seq.*; *Journal of Comparative Neurology*, 1916, Vol. 26, p. 1.

in the metabolic sequence conditioning development. This is suggested not only by the fact that autolysis typically leads to structural breakdown and cytolysis, but also and more especially by its being characteristically furthered by many of the general cytolytic and parthenogenetic agents, such as lipoid-solvents, acids and high temperatures. Ether, chloroform and alcohol greatly accelerate the autolysis of liver-cells and shorten the latent period of the process;[1] and acids, including CO_2, are well known to have the same effect.[2] Chiari attributes the effect of lipoid-solvents to an increase of protoplasmic permeability, allowing readier diffusion of enzymes and the other substances concerned. There is also evidence that autolytic processes underlie various structural and other physiological changes, some of which are of a kind frequently met with in development, *e. g.*, atrophic changes[3] (to which are related normal regressive processes like the involution of the uterus and the resorption of

[1] Chiari, *Arch. f. exper. Path. u. Pharm.*, 1908, Vol. 60, p. 256; *cf.* also Yoshimoto, *Zeitschr. f. physiol. Chem.*, 1908, Vol. 58, p. 341.

[2] *Cf.* Hedin and Rowland, *Zeitschr. f. physiol. Chem.*, 1901, Vol. 32, p. 241; Schryver, *Biochemical Journal*, 1906, Vol. 1, p. 123; Arinkin, *Zeitschr. f. physiol. Chem.*, 1907, Vol. 53, p. 192; Bellazi, *ibid.*, 1908, Vol. 57, p. 389; Yoshimoto, *ibid.*, 1908, Vol. 58, p. 341; Bradley, *cf.* footnote page 153. Bellazi and Yoshimoto describe experiments with CO_2. *Cf.* also Lacqueur, *Zeitschr. f. physiol. Chem.*, 1912, Vol. 79, p. 82; Lacqueur finds that carbon dioxide promotes and oxygen checks the autolysis of liver-cells. According to Lyon and Shackell (*Journal of Biological Chemistry*, 1909, Vol. 7, p. 371) acetic acid promotes the autolysis of sea-urchin eggs.

It should be noted that in the activation of eggs by acid the visible effects of the treatment do not appear at once, but only after the eggs have been returned to normal sea-water; this shows that for the initiation of the membrane-forming process *time* is required, representing possibly the latent period of the enzyme action. Hedin's observation that a *temporary* treatment of various tissues with acetic acid greatly increases the subsequent rate of autolysis—*i. e.*, the autolysis is decidedly more rapid in previously treated than in untreated portions of tissue under otherwise similar conditions, and even in presence of alkali—may have a bearing here (*Cf.* Hedin, *Festkrift O. Hammarsten*, Upsala, 1906; also Rhodin, *Zeitschr. f. physiol. Chem.*, 1911, Vol. 75, p. 197). Hedin ascribes this result to the destruction of an inhibitory substance by the acid. This suggests the possibility that in the activation of eggs by acid the deciding factor may be the destruction of an antibody (anti-protease?) in the egg-cortex; an activation so induced would be irreversible (as seems in fact to be the case). This view, however, does not seem consistent with the observed relations between concentration of acid and time of action, as described above (see footnote on page 132). The question as to the precise conditions of acid-activation is evidently an open one.

[3] *Cf.* Jacoby, *Zeitschr. f. physiol. Chem.*, 1900, Vol. 30, p. 174.

larval structures in metamorphosis), and the translocation of reserve materials; the utilization of tissue-proteins in starvation also appears to be dependent upon autolysis.[1]

The recent studies of Bradley and his associates have thrown an interesting light upon the conditions under which acid promotes autolysis of liver-cells.[2] Apparently the acid does not act simply by rendering the reaction favorable to the activity of the cell-proteases, for increasing the acidity does not necessarily increase the hydrolysis of all proteins;[3] moreover certain foreign proteins like gelatine or peptone are readily digested in neutral or slightly alkaline solution in which the liver-proteins resist digestion. It would appear that the intracellular proteases are capable of activity under these conditions, provided they are furnished with the appropriate substrate. The essential action

[1] *Cf.* Lane-Claypon and Schryver, *Journal of Physiology*, 1904, Vol. 31, p. 169; Schryver, *Biochemical Journal, loc. cit.* In "Senescence and Rejuvenescence," Chapter 2, Child calls attention to the general importance of the process of reduction or regression during development and regeneration. Regarded in the purely chemical sense, regression is the reverse of construction; strictly speaking, both processes are constantly and simultaneously at work in any living organism; it is only when regression exceeds construction that the substance of the organism undergoes visible decrease. Local regressive changes of this kind appear always to accompany regeneration; the translocation of material from one part to another is a necessary condition of the process; and such evidence as we possess indicates that autolysis is an important factor in any local structural breakdown which furnishes the material required for construction elsewhere (*cf.* Schryver's observations on the increased rate of autolysis in the livers of fasting animals, *loc. cit.*). It must be admitted, however, that this evidence is deficient in many respects (*cf.* Morse, *Amer. Journ. of Physiol.*, 1914, Vol. 36, p. 145), and it seems likely that the essential factors in the disintegration as well as in the building up of structure are unknown at present. The possible rôle of autolysis in regeneration has been discussed recently by Loeb ("The Organism as a Whole from the Physicochemical Viewpoint," Putnams, 1916, p. 178); if autolysis is a factor in regeneration, we may safely assume that it is important in developmental processes in general, including the development of the organism from the egg, and in this case probably from the very first, as suggested in the hypothesis put forward in the text.

[2] Bradley and Morse, *Journal of Biological Chemistry*, 1915, Vol. 21, p. 209; Bradley, *ibid.*, 1915, Vol. 22, p. 113; Bradley and Taylor, *ibid.*, 1916, Vol. 25, pp. 261, 363.

[3] The liver proteins are very sensitive to increase of acidity (Bradley and Taylor, *loc. cit.*, 1916, p. 262), but foreign proteins added to the autolyzing mixture may or may not show increased digestion under these conditions; *e.g.*, the digestion of egg albumin is greatly increased by acid, while that of casein and peptone remains unaltered; $MnCl_2$ promotes the digestion of liver-proteins, but not that of egg-albumin.

of the acid consists rather in altering the character of the normal proteins of the cell, and rendering these a favorable substrate for the proteases present. The autolytic process is thus activated by the addition of acid; the increase in the rate and extent of autolysis is roughly proportional to the amount of acid added.[1]

If this interpretation is applied to the case of the activation of the starfish egg by butyric acid, certain interesting possibilities appear. The acid appears to act by combining chemically, as already seen. If we assume that it forms a combination with some structure-forming protein, which then becomes hydrolyzable by some enzyme present, the above facts appear in a clearer light. The transformation of a certain definite quantity of this protein into substrate for the enzyme would then form the condition for complete activation. The rate of this transformation would correspond to the rate of the activation-process and would be proportional to the concentration of acid. We suggest, therefore, that acids cause activation by forming a hydrolyzable combination for some intracellular enzyme, probably one of well-defined specificity. The activating influence of high temperatures also becomes intelligible on this hypothesis, and may be referred to the production of an acid (*e. g.*, lactic) which then combines to form the hydrolyzable substrate; the similarity in the time-relations of heat-activation and acid-activation is thus explained. The autolysis following this combination would constitute the first step in development.

What is most significant from the standpoint of the general physiology of development is the evidence that a structural modification, in itself apparently simple, is sufficient to alter the whole course of egg-metabolism in a definite and predetermined manner. We must recognize that development is essentially the expression or outcome of a complex chain or nexus of metabolic processes, which is released by the activating reaction. Evidently these processes include chemical interactions and syntheses of a highly diversified and specific kind; these latter determine the specific character of the development in any instance. Regarding their precise nature we are almost completely ignorant at present. But however complex and unanalyzable the whole

[1] Bradley and Taylor, *loc. cit.*, 1916, p. 274.

developmental sequence may appear, its initiation is almost certainly dependent upon some slight and non-specific and primarily structural alteration in the egg-system.

In the orderly progress of development from one stage to the next we must similarly assume at every stage a similar interdependence between the existing structural conditions and the rate and character of the form-determining as well as other metabolism. Each step in advance is thus largely a consequence of the immediately preceding structural modification, and in its own turn furnishes the structural conditions determining the next step. From this point of view the unification of the whole developmental sequence would seem to be conditioned, at least in its main features, by specific *structural* peculiarities, represented at the beginning of development by the inherited organization of the germ. This determines at the start—assuming normal external conditions—the course of the ensuing transformations, and hence the special kind of structure arising at each successive stage of development. Structure, however, is not alone to be considered; the germ is a metabolizing system, and the soluble and diffusible (non-structural) materials formed in metabolism undoubtedly also play an essential part, probably chiefly of a controlling or regulative kind (*e. g.*, as hormones). And since throughout development there is always this reciprocal interdependence between structure and metabolism, it is clear that such materials—which presumably change continually in their character as development proceeds—must influence at every stage the nature of the metabolic transformations and hence of the organized structure which is being built up. It seems further probable that the destruction or removal of inhibiting or injurious or anticatalytic compounds, as well as the formation and activation of specific catalysts (enzymes), is concerned in the developmental process from the very first.[1] In brief, the metabolism of the developing germ must be regarded as differing not qualitatively but only in certain of its quantitative aspects—especially in the predominance of growth and formative processes—from that of the adult organism.

[1] This is indicated (*e.g.*) by the existence in the sea-urchin egg of an antibody for the sperm-agglutinin or "fertilizin" (F. R. Lillie, *Journal of Experimental Zoölogy*, 1914, Vol. 16, p. 544).

As Child has expressed it, one chief problem in the physiology of development is to determine how metabolism produces structure.[1] The converse problem of how structure influences metabolism is equally important. The course of structural modification as development proceeds is orderly; to this must correspond a similarly orderly alteration in the character of the chemical transformations which furnish at the same time both the energy and the material for development. Another consideration is important here. Corresponding to the increasing structural diversity with advance in development there is an increasing chemical diversity; of this we have already ample evidence; hence the need for special mechanisms of integration and coördination, both structural and chemical, must become greater with each advance in development. Presumably the development of these mechanisms runs parallel with the development of the organism as a whole. The continued and stable existence of the latter at any stage in its life-history—of the embryo as well as of the adult—is in fact contingent upon the uninterrupted and adequate working of these regulatory processes. In this respect also the developing germ differs in no essential manner from the adult organism. The physiological problems presented by the organism at different developmental stages differ in degree rather than in kind. From the egg to the embryo and from the embryo to the adult the transitions are continuous; the physiology of maintenance and the physiology of growth and inheritance are at bottom the same.

[1] *Journal of Morphology, loc. cit.*, p. 193.

TABLE IV.

OPTIMUM TIMES OF EXPOSURE TO .006 *n* BUTYRIC ACID IN SEA-WATER AT DIFFERENT TEMPERATURES.

Temperature.	Number of Lot and Date.	Range of Exposures.	Optimum Exposures with Results.	Exposures Next Above and Next Below Optimum with Results (% Blastulæ).
		Minutes	*Min. % blastulæ*	
8°	2, June 9	3–54	35–40 (85–90)	30 m. (70–80); 46 m. (60–70)
	6, " 12	4–48	36–40 (85–90)	32 m. (70–80); 44 m. (65–75)
	9, " 16	3–45	30–40 (?)	27 m. (?); 45 m. (?)
	15, " 20	3–36	21–27 (20–35)	18 m. (15–20); 30 m. (few)
	23, " 26	3–36	21–24 (50–60)	18 m. (30–40); 27 m. (30–40)
10°	3, June 9	3–35	26–30 (*ca.* 95)	22 m. (80–90); 35 *m.* (*ca.* 90)
	6, " 12	4–48	28–36 (80–90)	24 m. (60–70); 40 m. (70–80)
	9, " 16	3–36	24–36 (*ca.* 50)	21 m. (40–50);
	15, " 20	3–33	*ca.* 18 m (40–50)	15 m. (35–45); 21 m. (35–45)
	23, " 26	3–30	18–21 (65–75)	15 m. (55–60); 24 m. (25–35)
12°	2, June 9	3–35	18–26 (*ca.* 95)	15 m. (80–90); 30 m. (*ca.* 90)
	9, " 16	3–36	21–24 (50–60)	18 m. (40–50); 27 m. (*ca.* 50)
	15, " 20	3–30	12–15 (50–60)	9 m. (10–15); 18 m. (20–30)
	22, " 26	2–22	8–12 (70–90)	6 m. (25–35); 14 m. (*ca.* 75)
14°	4, June 10	2–26	22–24 (70–80)	20 m. (50–60); 26 m. (*ca.* 70)
	10, " 17	1–12	>12 m. (optimum not reached)	12 m. (15–20);
	12, " 19	2–22	*ca.* 14 m. (65–70)	12 m. (30–40); 16 m. (40–50)
	14, " 20	2–22	*ca.* 14 m. (70–80)	12 m. (65–75); 16 m. (*ca.* 50)
	22, " 26	2–20	*ca.* 10 m. (80–85)	8 m. (60–70); 12 m. (*ca.* 50)
16°	4, June 10	2–22	18–20 (75–85)	16 m. (70–80); 22 m. (70–80)
	10, " 17	1–12	>12 (optimum not reached)	12 m. (60–70)
	12, " 19	2–18	*ca.* 10 (85–90)	8 m. (45–50); 12 m. (70–80)
	14, " 20	2–18	*ca.* 10 (85–90)	8 m. (35–40); 12 m. (65–70)
	22, " 26	2–18	6–8 (70–85)	4 m. (*ca.* 50); 10 m. (15–20)
18°	1, June 8	2–24	10–12 (80–90)	8 m. (65–75); 14 m. (60–70)
	2, " 9	3–26	9–12 (90–95)	6 m. (65–70); 15 m. (20–30)
	4, " 10	2–18	12–14 (75–90)	10 m. (50–60); 16 m. (*ca.* 80)
	10, " 17	1–12	9–10 (70–85)	8 m. (65–76); 11 m. (50–60)
	12, " 19	2–14	*ca.* 8 (85–90)	6 m. (65–75); 10 m. (40–50)
	14, " 20	2–14	6–8 (70–80)	4 m. (*ca.* 10); 10 m. (40–50)
	21, " 24	1–9	5–6 (80–90)	4 m. (60–70); 7 m. (70–80)
20°	5, June 11	1–12	5–6 (70–90)	4 m. (40–50); 7 m. (60–70)
	7, " 12	1–10	8–9 (90–95)	7 m. (60–70); 10 m. (*ca.* 90)
	11, " 17	1–11	6–7 (*ca.* 95)	5 m. (80–90); 8 m. (80–90)
	13, " 19	1–6	3–4 (*ca.* 95)	2 m. (30–35); 5 m. (70–80)
	18, " 23	2–12	*ca.* 4 (*ca.* 50)	2 m. (20–25); 6 m. (25–35)
	21, " 24	1–8	3–4 (90–95)	2 m. (15–20); 5 m. (80–90)
22°	5, June 11	1–12	3–4 (*ca.* 90)	2 m. (2–3); 5 m. (20–25)
	7, " 12	1–8	6–7 (*ca.* 95)	5 m. (70–80); 8 m. (*ca.* 90)
	11, " 17	1–10	*ca.* 4 (*ca.* 95)	3 m. (80–90); 5 m. (*ca.* 90)
	13, " 19	1–5	2–3 (80–90)	1 m. (1–2); 4 m. (5–10)
	16, " 22	½–4	3–3½ (65–75)	2½ m. (60–70); 4 m. (50–60)
	21, " 24	½–3½	2–3 (75–90)	1½ m. (35–40); 3½ m. (60–70)

Temperature.	Number of Lot and Date.	Range of Exposures.	Optimum Exposures with Results.	Exposures Next Above and Next Below Optimum with Results (% Blastulæ).
		Min.	*Min.* % *blastulæ*	
	5, June 11	1–12	2 m. (60–65)	1 m. (0); 3 m. (15–20)
24°	7, " 12	1–6	3–4 (90–95)	2 m. (30–35); 5 m. (40–50)
	16, " 22	½–3½	2–2½ (80–85)	1½ m. (*ca.* 50); 3 m. (10–20)
	17, " 22	½–3	1½–2 (80–95)	1 m. (25–35); 2½ m. (40–50)
	21, " 24	½–3	1½–2 (*ca.* 90)	1 m. (5–10); 2½ m. (50–60)
26°	8, June 13	1–5	*ca.* 2 (few: 5–10)	1 m. (*ca.* 5); 3 m. (0)
	17, " 22	½–3	1–1½ (70–80)	½ m. (<1); 2 m. (10–15)
	18, " 23	½–3	1 (20–30)	½ m. (1–2); 1½ m. (5–10)
	19, " 23	½–2½	1 (30–40)	½ m. (<1); 1½ m. (0)
	20, " 24	½–2	1 (80–90)	½ m. (5–10); 1½ m. (30–35)
28°	8, June 13	1–4	1 (*ca.* 5)	2 m. (0)
	18, " 23	½–2	½ (10–15)	1 m. (0)
	19, " 23	½–2	½ (25–35)	1 m. (0)
	20, " 24	½–2	½ (80–90)	1 m. (<1)